September 13, 2000

Betty Moran
Manager, Olefins Panel
American Chemistry Council
1300 Wilson Blvd.
Arlington, VA 22209

Dear Ms. Moran:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for the Crude Butadiene C4 category, submitted May 9, 2000. I commend the ACC Olefins Panel for their commitment to the HPV Challenge Program.

EPA reviews test plans and robust summaries to determine whether the reported data and test plans will adequately characterize each SIDS endpoint. On its Chemical RTK HPV Challenge Program website EPA has provided guidance for determining the adequacy of data and preparing test plans used to prioritize chemicals for further work.

EPA will post this letter and the attached Comments on the Chemical RTK web site within the next few days. As noted in the comments, we ask that the Panel advise the Agency, within 60 days of the posting on the Chemical RTK website, how it intends to pursue its activities on these chemicals. Please respond either by email (oppt.ncic@epa.gov, hpv.crtk@epa.gov, or chem.rtk@epa.gov)or by regular mail to:

Carol Browner, Administrator
US Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116
Attention: Chemical Right-to-Know Program

EPA will post your response on the Chemical RTK website.

If you have any questions about this response, please contact Richard Hefter, Chief of the HPV Chemicals Branch, at 202-260-3470. Submit general questions about the HPV Challenge Program through the Chemical RTK web site comment button or through the TSCA Assistance Information Service (TSCA Hotline) at (202) 554-1404. The TSCA Hotline can also be reached by e-mail at tsca-hotline@.epa.gov.

I thank you for your submission and look forward to your continued participation in the HPV Challenge Program.

Sincerely,

/s/

Oscar Hernandez, Director
Risk Assessment Division

Attachment

cc: W. Sanders
C. Auer
N. Patel
A. Abramson

EPA Comments on Chemical RTK Challenge Submission: Crude Butadiene C4s

SUMMARY OF EPA COMMENTS

The sponsor, the CMA (now ACC) Olefins Panel's HPV Implementation Task Group, submitted Robust Summaries to EPA and a Test Plan and Category Justification dated May 9, 2000. EPA posted the submission on the ChemRTK website on May 19, 2000. The proposed information-gathering plan is for 1,3-Butadiene (CAS No. 106-99-0) and eleven CAS-numbered petroleum process streams containing 1,3-butadiene, all considered by the sponsor to constitute a Crude Butadienes category.

EPA has reviewed this submission and has reached the following conclusions:

1. The submission comprised an acceptable category submission and test plan overall. While the descriptive material has some confusing aspects and inconsistencies, the sponsor's intentions are adequately clear.

2. As a rule, measured physicochemical property data should be provided, both to characterize a substance and to provide inputs to transport/distribution models; these should be available from published sources on the generally well-known butadiene mixture components. The Test Plan for physicochemical properties suggests that all data on these properties will be derived from EPIWIN calculations.

3. EPA concurs that these gaseous substances are not amenable to environmental fate testing under the Challenge Program. The sponsor plans to use the EQC Level I steady state model to develop distribution data on individual mixture components. However, recent experience in the use of distribution models has caused EPA to conclude that the EQC Level III model gives better results.

4. EPA is concerned that the sponsor's approach to characterizing "full-range butadiene concentrate" may be inadequate. Although the plan to use data from butadiene, benzene, and other related HPV Challenge categories to address the toxicity of this substance may be sufficient, details are sparse, and EPA has not yet received the Panel's test plans for related categories. Thus not all the information necessary to evaluate this part of the test plan may be available. EPA reserves judgement on this point until test plans are available for all the streams containing aromatics.

5. EPA believes further acute inhalation toxicity testing will not be informative for the Challenge Program, as existing limit-test data on 1,3-butadiene and the midrange test substance show no lethality. If the sponsor needs this type of testing for other purposes, EPA suggests that a limit test be performed.

6. The 10% butadiene test substance should be properly characterized. Care should be taken to ensure that the 10% butadiene test substance has a similar component profile to the mid-range butadiene test substance in order to assure comparability of results. The 10% butadiene test substance should be fully characterized by listing all components in the stream and their percentages.

7. The OECD SIDS program's reproductive toxicity analysis for 1,3-butadiene is not yet complete. Resolution of this endpoint will have an impact on this category proposal. EPA believes it is appropriate to understand the outcome of the OECD SIDS process for 1,3-butadiene to better inform the reproductive hazard evaluation of 100% butadiene and the proposal to test 10% butadiene.

8. EPA is concerned that the reproductive/developmental toxicity results for 1,3-butadiene and the 10% butadiene test substance be reasonably comparable. Interpolating the reproductive and developmental toxicity results to the mid-range butadiene stream requires a comparison between the 100% butadiene and the 10% butadiene mixture. There needs to be a reasonable basis for comparison, which may mean comparable protocols and/or test species and a similar mixture component profile as discussed under #6 and #7 above.

9. The mouse appears to be the preferred species for health effects testing On the basis of repeat-dose, mutagenicity and developmental toxicity studies, mice appear more sensitive than rats to 1,3-butadiene. The sponsor has not indicated a species preference for the proposed testing. EPA suggests that the sponsor consider conducting the remaining *in vivo* health effects studies in mice. It would also be helpful to know what test protocols will be used.

10. A few robust study summaries were considered inadequate. Details appear in the text under "Specific Comments on Robust Summaries."

11. As with all category proposals, the outcome of the proposed testing may change the approach to the category as originally proposed.

EPA is requesting that the Sponsor advise the Agency within 60 days how it intends to pursue activities on the proposed test plan.

EPA COMMENTS ON THE CRUDE BUTADIENE C4 CHALLENGE SUBMISSION

EPA's comments are organized as follows: General; Category Description; Category Justification; Test Plan; Specific Comments on Robust Summaries.

General

The sponsor supplied a complete package. The test plan was thorough and included a flow diagram that showed the complex petroleum refining processes and how the proposed category of butadiene streams fits into the larger picture, although the text was not perfectly matched to the diagram. There was some inconsistency in nomenclature in the text. The robust summaries were reasonably well organized

Category Description

General Clarity

The description was sometimes confusing and difficult to assimilate. The process diagram is helpful but would be more so with additional labeling to reflect the description in the text.

Some examples of confusing usage:

> There is no process stream in the Flowsheet that is identified as the "full-range butadiene concentrate" referred to frequently in the text.
>
> "Crude butadiene" is defined in section II. A as "[t]hose mixtures containing 10 to 92% 1,3-butadiene...", but in the title to section C.1 of Appendix I, "crude butadiene" appears to be synonymous with "butadiene concentrate", which contains 10-80% 1,3-butadiene.
>
> Again in Appendix I, it is unclear if "C4 butadiene concentrate" is different from "butadiene concentrate" mentioned in Sections C.1 and C.2.

Despite these shortcomings, EPA agrees that the important point that emerges is the existence of different streams containing a range of possible 1,3-butadiene concentrations.

The category is simply 1,3-butadiene plus the three generic streams (full-range butadiene concentrate, butadiene concentrate, and high butadiene heavy ends) associated with the ethylene production process. Some difficulty arises with the fact that, except for 1,3-butadiene, any of the nominal CAS-numbered category members may be associated with any of the three streams, according to the sponsor. However, EPA acknowledges that petroleum refining processes and products, as well as the chemical composition of such complex mixtures, do not lend themselves to a simple, straightforward assignment to a "traditional" category in the U.S. HPV Challenge Program.

Category Justification

The submission presents a case for considering Crude Butadiene C4 substances as a category. EPA believes the presentation adequately supports this proposal.

This is the first example of a submission to the Challenge Program that is based on a) process streams, and b) the presence of a single well-characterized component of all the streams, 1,3-butadiene, that the submitter proposes will determine the toxicity of all the mixtures listed in the proposal. The submitter also plans to review data on other mixture components that already exist or are in development under

other test plans under the HPV Challenge or other programs "to assist...in determining whether butadiene is the most biologically active component of the Crude Butadiene C4 streams."

EPA believes that, for these mixtures, estimating toxicity on the basis of their 1,3-butadiene content is a reasonable proposal for the health effects portion of the SIDS.

However, EPA is concerned that the sponsor's approach to characterizing "full-range butadiene concentrate" may be inadequate. Although the plan to use data from butadiene, benzene and other higher-than C_4 constituents, and other related HPV Challenge categories to address the toxicity of this substance may be sufficient, it lacks details and sponsors have not yet submitted test plans for related categories. Thus reviewers may not have all the information necessary to evaluate this part of the test plan.

Test Plan

Chemistry (melting point, boiling point, vapor pressure, water solubility, and partition coefficient). The sponsor plans to develop data on individual mixture components, using EPIWIN, and provide ranges of values for the different streams. However, EPA emphasizes that measured data shoiuld be provided for this purpose. With chemicals such as these mixture components that are well characterized or are being characterized in OECD SIDS and other programs, acceptable literature data may be available for many physicochemical endpoints and should be included wherever possible. (Generally, the log P value can be calculated for chemical classes that have been validated for the calculation.)

Fate (photodegradation, stability in water, biodegradation, and transport/distribution). EPA concurs that these substances are not amenable to environmental fate testing. The sponsor plans to develop distribution data on individual mixture components, using available models. The test plan cites the EQC Level I steady state model as one accepted by EPA in its guidance for the Challenge program. This attribution is correct. However, recent experience in the use of distribution models has caused EPA to conclude that the EQC Level III model gives better results.

As stated above under Chemistry, as a rule measured values rather than calculated physicochemical values should be used as inputs for the models.

Additionally, in situations of this kind, reviewers would find it useful to know which components of the streams sponsors intend to model.

Health Effects (acute toxicity, repeat dose toxicity, genetic toxicity, and reproductive/developmental toxicity). EPA agrees with the proposed plan to conduct all the health effects studies on a low-end (e.g., 10% butadiene) mixture. However, EPA does not agree that an acute inhalation toxicity study is informative for the purposes of the U.S. HPV Challenge Program (see item 4 below).

EPA does have some concerns about details provided in the proposal:

(1) EPA assumes that the 10% butadiene test substance will come from either the "crude butadiene/butadiene concentrate" or "heavy ends" streams as identified in Table 2 of the Test Plan. Whichever is used, it does not correlate closely to the third stream ("full-range butadiene concentrate") which is the only stream with components higher than C4. More importantly, the two "C4 streams" appear to have different components (and amounts of each component). Care should be taken to ensure that the 10% butadiene test substance contains the same non-butadiene components as the mid-range butadiene test substance in order to attribute any effect (or lack thereof) to 1,3-butadiene. This requires that the 10% butadiene test substance be fully characterized by listing all components in the stream with their percentages.

(2) Assuming that a 10% butadiene test substance is used in a combined test protocol as proposed, in order to interpolate the reproductive/developmental toxicity results to the mid-range butadiene stream a comparison between the 100% butadiene and the 10% butadiene stream needs to be made. There needs to be a reasonable basis for comparison which may mean the need for comparable protocols (see item 3 below)and/or test species (see item 5 below).

(3) The reproductive toxicity of 1,3-butadiene has not yet been resolved in the OECD SIDS program. Resolution of this issue will affect this category proposal. EPA will reserve comment on this endpoint for 1,3-butadiene until the sponsor develops this argument more fully by incorporating the OECD SIDS assessment findings. EPA believes whatever test protocol(s) are identified/used will affect this category analysis as outlined in item #2 above.

(4) For the purposes of the U.S. Challenge Program, EPA believes that an acute inhalation toxicity study may not be informative. Available test results show that pure butadiene and the mid-range butadiene did not cause lethality at or above the limit of 5 mg/L for such tests and differences in proportions of constituents between the midrange and 10% butadiene mixtures are unlikely to result in significant acute toxicity of the latter. However, if the sponsor needs this type of testing for other purposes, EPA suggests a limit test.

(5) On the basis of repeat-dose, mutagenicity and developmental toxicity studies presented in the Test Plan, as well as many other examples in the literature, it appears that mice are more sensitive than rats to 1,3-butadiene. EPA suggests that the sponsor consider conducting the remaining *in vivo* health effects studies in mice. With test plans of this kind it would be useful to know what test protocols will be used.

Ecological Effects. Because these substances are gases that are rapidly lost from water, EPA accepts the sponsor's proposal to calculate toxicity data for selected mixture components, using ECOSAR or an equivalent aquatic toxicity estimation program. (Gases having properties different than those in this category could be found to need testing; methods are available to address such situations.) Note that when using ECOSAR to predict toxicity values, the CLOGP version of the octanol/water partition coefficient (Log Kow) should be used instead of the Log Kow that EPIWIN automatically enters. This is because ECOSAR values were developed using CLOGP.

Specific Comments on Robust Summaries

The only robust summaries submitted described health effects studies. Seventeen studies were submitted. EPA evaluated each acute, repeat dose, and genotoxicity robust summary and determined that all but one were adequate summaries for the purposes of the U.S. HPV Challenge Program. The robust summary for irritation screening in rabbits with CAS # 68955-28-2, while not among the required SIDS endpoints, was reviewed for its relevance to the test plan.

The *in vivo* mouse dominant lethal assay summary was considered inadequate because the basis for dose selection is not stated. Since there were both positive and negative results (depending upon time point of evaluation), this is considered a critical omission.

Acute Toxicity. Two robust summaries describing acute inhalation toxicity studies, one with CAS # 68955-28-2 (45% butadiene, 20% butanes, and 30% butenes) and one with CAS # 106-99-0 (1,3-butadiene) were reviewed.

The study performed with the 45% butadiene content substance reported a 4-hour LC50 of > 5300 mg/m^3 (5.3 mg/L) in rats (the limit test for inhalation studies is 5 mg/L, or 5000 mg/m^3). Therefore the test mixture used can be considered a low acute inhalation toxicity hazard. (NOTE: The summary lists OECD Test Guideline (TG) 402 as the study protocol but it is likely that OECD TG 403 was used; 402 is for dermal studies).

The 100% butadiene study was not run under GLP conditions. It describes a study in rats and mice showing a 4-hour LC50 of 285 mg/L (much higher than 5 mg/L limit test) in rats and a 2-hour LC50 of 270 mg/L in mice. No experimental details were provided and data quality cannot be determined; thus the study summary is inadequate.

Taken together, these studies show that these gases present a low acute inhalation toxicity hazard and there appears to be no significant difference in response. EPA believes that the difference in proportions of constituents between the midrange and 10% butadiene mixtures is unlikely to result in significant acute toxicity of the latter.

Repeat Dose Toxicity. Three robust summaries describing repeat dose studies were reviewed, two with CAS # 106-99-0 and one with CAS # 68955-28-2.

A subchronic inhalation study in B6C3F1 mice was conducted by the National Toxicology Program. The purpose of the study was to establish doses for a 2-year bioassay. Doses of 0, 625, 1250, 2500, 5000, or 8000 ppm (0,1250, 2500,10000, 16000 mg/m^3) 1,3-butadiene were administered to mice (10/sex/dose) for 6 hours/d, 5 days/wk, for 14 weeks. Mortality was observed at the two higher doses. A NOAEL was established at 1250 ppm based on reduced body weight gains. Histopathological examinations were performed on controls and high dose animals and no effects were observed.

The second 1.3-butadiene summary describes a subchronic inhalation study in which Sprague-Dawley rats (40/sex/dose) were given doses of 0, 1000, 2000, 4000, or 8000 ppm 1,3-butadiene for 6 hours/d, 5 days/wk, for 13 weeks. There were no mortalities. A NOAEL was established at > 8000 ppm and the only exposure-related findings were increased salivation (females after 8 weeks' exposure) and decreased grooming (males after 10 weeks' exposure). Histopathological examinations were performed on controls and high dose animals and no effects were observed.

In the third study, Fisher 344 rats (5/sex/dose) were exposed to the CAS # 68955-28-2 via inhalation 6 hours/d, for nine days over a 12-day period at doses of 0, 1110, and 11,140 ppm). There were no significant effects in the rats over the course of the study. The only effect observed was nasal discharge in some rats in both treated groups, with a greater incidence in the high dose group. Thus, the NOAEL for this study was >11,140 ppm.

Taken together, these studies demonstrate the species differences in response (mortality and reduced body weight gain) to 1,3-butadiene exposure (mice being more susceptible than rats). The two rat studies suggest that 100% butadiene and a mid-range butadiene (45%) produce similar results; however, the test protocols used were quite different (13 weeks of exposure versus 12 days). There are no comparison data for mice, which were tested only with 1,3-butadiene itself.

Genotoxicity Studies. Seven robust summaries were reviewed, for genotoxicity and related effects (cell transformation) and all were properly summarized.

a) 1,3-Butadiene (CAS# 106-99-0)

The *Salmonella typhimurium*/mammalian microsomal (Ames) assay showed 1,3-butadiene to be a weak mutagen with activation (positive in one of four strains tested with uninduced rat and mouse liver enzymes and induced rat liver enzymes).

The *in vivo* micronucleus assay in male rats and female mice by inhalation showed a positive response by inducing micronuclei in the peripheral blood and bone marrow erythrocytes in mice at all dose levels (50, 200, or 500 ppm via inhalation 6 hours/day for 5 days), whereas there was a negative response in rats given the same treatment.

b) Butadiene concentrate (CAS# 68955-28-2) - (Gases (petroleum) light steam-cracked, butadiene conc.) Approximately 45% 1,3-butadiene, 20% butanes and 30% butenes.

The *Salmonella typhimurium*/mammalian microsomal (Ames) assay was negative in all strains tested with and without metabolic (rat only) activation.

The L5178Y $TK^{+/-}$ mouse lymphoma gene mutation assay *in vitro* assay showed a positive response without metabolic activation and a negative response with metabolic activation (rat liver enzyme preparations were used for activation).

The *in vivo* micronucleus assay in male and female mice by inhalation showed a positive response by inducing micronuclei in the bone marrow erythrocytes in mice at all dose levels (10, 780; 20, 670; or 35, 340 ppm via inhalation 2 hours/day for 2 days).

The unscheduled DNA synthesis (UDS) assay in rat hepatocytes *in vitro* showed a weak positive response.

The cell transformation assay in BALB/3T3 cells *in vitro* showed a negative response.

Taken together, the data presented by the sponsor show that 1,3-butadiene appears to affect the chromosome and that mice appear to be more sensitive for this endpoint than rats. Comparing the results (mouse only) of the 45% butadiene micronucleus assay with the 100% butadiene micronucleus assay suggests that the butadiene-based category proposal has merit for this endpoint.

Reproductive Toxicity Studies. The Test Plan indicates that adequate data are not yet available for 1,3-butadiene, but they should be addressed in the OECD SIDS program. However, data are presented from two studies (spermhead morphology and dominant-lethal studies, both in mice) on 1,3-butadiene that are informative in addressing reproductive toxicity. In both cases, the robust summaries are considered inadequate because the basis for dose selection is not provided. EPA believes this inadequacy is not

critical for the spermhead morphology study because the results were positive.

> A sperm morphology assay in which 20 CD-1 male mice were exposed to 0, 200, 1000, 5000 ppm 1,3-butadiene via inhalation 6 hours/day for 5 days showed a positive response (slight but dose-related increase in abnormal sperm heads with dose).
>
> The *in vivo* mouse dominant lethal assay in males exposed by inhalation does not indicate the basis for the dose selection. Since the results were both positive and negative (depending upon the time point of evaluation), this is a critical omission.

EPA believes it is appropriate to understand the outcome of the OECD SIDS process for 1,3-butadiene to better inform the reproductive hazard evaluation of 100% butadiene and the proposal to test 10% butadiene.

Developmental Toxicity Studies. The robust summaries of the two inhalation developmental toxicity studies (in rats and mice, both with 100% 1,3-butadiene) are adequate. However, EPA has the following comments:

> The LOAEL and the basis for that effect level should be specifically cited in the Results section for the robust summaries for both developmental toxicity studies.
>
> In the robust summary for the mouse study:
>
> > (1) signs of developmental toxicity in mice (decreases in fetal weights, increases in fetal variations) were reported at concentrations of 200 and 1000 ppm. The decreases in fetal weights were reported as being significantly reduced while the increases in fetal variations were reported as being 'increased'. There needs to be some mention of whether or not this increase was statistically significant; and
> >
> > (2) in the Results section, fetal weights were reported as being reduced in both males and females, while in the Conclusions section, this endpoint was reported as a "slight statistically significant decrease in male fetal weight". This needs to be clarified as to whether the reduction in female fetal weight was significant.

Signs of developmental toxicity in mice (significant decreases in fetal body weights and increases in fetal variations) were observed at 200 ppm and 1000 ppm. No signs of developmental toxicity were observed in rats at concentrations as high as 1000 ppm. As in the case of repeat-dose and mutagenicity studies, it appears that mice are more sensitive than rats to 1,3-butadiene.

Followup Activity

EPA requests that the Sponsor advise the Agency within 60 days how it intends to pursue activities on the proposed test plan.